

WHAT IS CLAIMED:

1. A method for producing an artificial chromosome, comprising:
introducing a DNA fragment into a cell, wherein the DNA
fragment comprises a selectable marker;
5 growing the cell under selective conditions to produce cells
that have incorporated the DNA fragment into their genomic DNA; and
selecting a cell that comprises a satellite artificial
chromosome [SATAC].
2. The method of claim 1, further comprising, isolating the
10 SATAC.
3. A SATAC produced by the method of claim 1.
4. The method of claim 1, wherein the DNA fragment
comprises a sequence of nucleotides that targets the fragment to the
heterochromatic region of a chromosome.
- 15 5. The method of claim 4, wherein the targeting sequence of
nucleotides is satellite DNA.
6. A cell containing an artificial chromosome, wherein the
artificial chromosome is produced by the method of claim 1.
7. The cell of claim 6, wherein the artificial chromosome is a
20 satellite artificial chromosome [SATAC].
8. An isolated substantially pure satellite artificial chromosome
[SATAC].
9. The SATAC of claim 8 that is a megachromosome,
comprising about 50 to about 450 megabases [Mb].
- 25 10. The SATAC of claim 9, comprising about 250 to about
400 Mb.
11. The SATAC of claim 9, comprising about 150 to about
200 Mb.
12. The SATAC of claim 9, comprising about 90 to about 120 Mb.

13. The SATAC of claim 9, comprising about 60 to about 100 Mb.

14. The method of claim 1, further comprising introducing a fragmentation vector, whereby megachromosomes in the cells of step B are reduced in size to resulting in cells that contain SATACs that are
5 about 15 to about 50 Mb.

15. The method of claim 14, further comprising selecting a cell that comprises a satellite artificial chromosome [SATAC] that comprises about 15 to about 50 Mb.

16. A cell containing an artificial chromosome, wherein the
10 artificial chromosome is produced by the method of claim 14.

17. A cell containing an artificial chromosome, wherein the artificial chromosome is produced by the method of claim 15.

18. The cell of claim 15, wherein the artificial chromosome is a SATAC comprising about 15 to about 50 Mb.

15 19. An isolated substantially pure satellite artificial chromosome [SATAC] that comprises about 15 to about 50 Mb.

20. The method of claim 1, further comprising isolating the SATAC from the cell.

21. The method of claim 1, wherein isolation is effected by:
20 isolating metaphase chromosomes;
staining the chromosomes with DNA sequence-specific dyes; and
separating the SATACs from other chromosomes in the cells by
flow cell sorter.

22. A method for producing an artificial chromosome,
25 comprising:

introducing a DNA fragment into a cell, wherein the DNA fragment comprises a selectable marker,
growing the cell under selective conditions to produce cells that have incorporated the DNA fragment into their genomic DNA,

selecting from among those cells, a cell that has a chromosome that comprises a *de novo* centromere.

23. The method of claim 22, further comprising isolating that cell with the chromosome that comprises the *de novo* centromere, and
5 growing the cell under conditions whereby a cell with a sausage chromosome is produced.

24. The method of claim 23, further comprising isolating the cell with the sausage chromosome; and growing the cell under conditions whereby a first SATAC is produced.

10 25. The method of claim 24, further comprising:
introducing a fragmentation vector that is targeted to the first SATAC; growing the cells; and selecting a cell that comprises a second SATAC, wherein the second SATAC is smaller than the first SATAC.

26. The method of claim 22, wherein the selected cell has a
15 dicentric chromosome comprising the *de novo* centromere.

27. The method of claim 22, wherein the selected cell has a formerly dicentric chromosome and a minichromosome comprising the *de novo* centromere.

28. The method of claim 22, wherein the selected cell has a
20 formerly dicentric chromosome.

29. A method for producing an artificial chromosome, comprising introducing a DNA fragment into a cell, wherein the DNA fragment comprises a selectable marker;

growing the cell under selective conditions to produce cells
25 that have incorporated the DNA fragment into their genomic DNA;

selecting from among those cells a cell that has produced a dicentric chromosome; and

growing that cell under selective conditions, whereby a cell that contains a chromosome comprising a heterochromatic arm is
30 produced.

30. The method of claim 29, further comprising selecting the cell with the chromosome comprising the heterochromatic arm and growing it in the presence of an agent that destabilizes the chromosome.

31. The method of claim 30, further comprising identifying cells
5 that contain a heterochromatic chromosome that is about 50 to about 400 Mb.

32. A method for producing a transgenic animal, comprising introducing a satellite artificial chromosome [SATAC] into an embryonic cell.

10 33. The method of claim 32, wherein the embryonic cell is a stem cell.

34. The method of claim 32, wherein the embryonic cell is in an embryo.

35. The method of claim 32, wherein the SATAC comprises heterologous DNA that encodes a therapeutic product.
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36. The method of claim 32, wherein the product is the cystic fibrosis transmembrane regulatory protein [CFTR], an anti-HIV ribozyme, or a tumor suppressor gene.

20 37. The method of claim 32, wherein the anti-HIV ribozyme is an anti-*gag* ribozyme, and the tumor suppressor gene is p53.

38. The method of claim 32, wherein the product comprises an antigen that upon expression induces an immunoprotective response against a pathogen in the transgenic animal.

25 39. The method of claim 32, wherein the product comprises a plurality of antigens that upon expression induce an immunoprotective response against a plurality of pathogens.

40. The method of claim 32, wherein the transgenic animal is a fish, insect, reptile, amphibians, arachnid or mammal.

510 37
 41. The method of claim 32, wherein the SATAC is introduced by cell fusion, microinjection, microcell fusion, electroporation, microprojectile bombardment or direct DNA transfer.

42. A transgenic animal produced by the method of claim 32.

5 43. A method of for producing a transgenic plant or animal, comprising:

introducing a DNA fragment into a cell, wherein the DNA fragment comprises a selectable marker;

growing the cell under selective conditions to produce cells
 10 that have incorporated the DNA fragment into their genomic DNA; and
 selecting a cell that comprises a minichromosome that is about 10 Mb to about 50 Mb that comprises the selectable marker and euchromatin;

isolating the minichromosome and introducing it into a plant
 15 or animal cell.

44. The method of claim 43, wherein: after selecting the cell, DNA encoding a gene product or products is introduced into the cell, and the cell is grown under selective conditions, whereby cells comprising minichromosomes comprising the DNA encoding the gene product(s) are
 20 produced.

45. The method of claim 2, wherein: after selecting the cell, DNA encoding a gene product or products is introduced into the cell, and the cell is grown under selective conditions, whereby cells comprising SATACS that comprise the DNA encoding the gene product(s) are
 25 produced.

46. A method for producing a transgenic plant, comprising introducing a satellite artificial chromosome [SATAC] into a plant cell; and culturing the cell under conditions whereby a plant is generated.

47. The method of claim 46, wherein the SATAC is introduced by protoplast fusion, microinjection, microcell fusion, electroporation, microprojectile bombardment or direct DNA transfer.

48. A method for producing a gene product(s), comprising
5 introducing a satellite artificial chromosome [SATAC] into a cell; and culturing the cell under conditions whereby the gene product(s) is (are) expressed.

49. The method of claim 48, wherein the gene product is produced by expression of a series of genes that encode a metabolic
10 pathway; and the SATAC comprises each of these genes.

50. A method for cloning a centromere from an animal or plant, comprising:

preparing a library of DNA fragments that comprise the genome of the plant or animal;

15 introducing the each of the fragments into mammalian satellite artificial chromosomes [SATACs], wherein:

each SATAC comprises a centromere from a different species from the selected plant or animal, and a selectable marker;

20 introducing each of the SATACs into the cells and growing the cells under selective conditions;

identifying cells that have a SATAC; and

selecting from among those cells any that have a SATAC comprising a centromere that differs from the centromeres in the original SATAC.

25 51. A cell line having the identifying characteristics of any of TF1004G19C5, 19C5xHa4, H1D3 and G3D5, which have been deposited at the ECACC under Accession Nos. 96040926, 96040927, 96040929, and 96040928, respectively.

30 52. A cell line, comprising a megachromosome that comprises about 50-400 Mb.

53. A cell line of claim 52, wherein the megachromosome comprises about 250 to about 400 Mb.

54. A cell line of claim 52, wherein the megachromosome comprises about 150 to about 200 Mb.

5 55. A cell line of claim 52, wherein the megachromosome comprises about 90 to about 120 Mb.

56. A cell line of claim 52, wherein the megachromosome comprises about 60 to about 100 Mb.

57. A method for gene therapy, comprising:
10 introducing a SATAC that comprises DNA therapeutic product into a target cell; and

introducing the resulting target cells into a host animal.

58. The method of claim 57, wherein the target cells are lymphocytes, stem cells or nerve cells.

59. The method of claim 43, wherein the minichromosome is the minichromosome present in the cell line EC3/7C5.

60. The method of claim 43, wherein the chromosome is the λ neo-chromosome in the cell line KE1 2/4.

61. An artificial chromosome produced by the method of claim 1
20 that comprises more euchromatin than heterochromatin.

62. The artificial chromosome of claim 61 that is between about 20 Mb and about 200 Mb.

63. The artificial chromosome of claim 61 that is between about 100 Mb and about 200 Mb.

add
A'
add B' >
add G1)